NOTES ON THE OCCURRENCE OF THE TRICHOPELTACEAE AND ATICHIACEAE IN NEW SOUTH WALES, AND ON THEIR MODE OF NUTRITION, WITH A DESCRIPTION OF A NEW SPECIES OF *ATICHIA*.

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(Plates xiii–xiv; ten Text-figures.)

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The Trichopeltaceae and Atichiaceae are two families of epiphyllous ascomycetes considered by the writer to form part of the sooty-mould complex. They are usually associated with other sooty-mould fungi, the most important of which are the species of the Capnodiaceae (Fraser, 1935*a*, 1935*b*). Both the Trichopeltaceae and the Atichiaceae are specialized for their environment but in very different ways. They do not appear to be closely related systematically.

The method of nutrition of the species of these families has not previously been established, most of the work which has been done on them being purely systematic.

The Trichopeltaceae.

a. Historical.

The species belonging to this family have a peculiar mycelium which is quite distinct from that of any other fungus. The hyphae, instead of being separate from each other, remain in contact to form a flat thallus, one cell thick and many cells wide. It resembles in structure the thallus produced by the algae of the family Trentipohliaceae, especially the epiphyllous genera *Phycopeltis* and *Cephaleuros*. The thallus may be circular, in which case growth takes place round the whole circumference, or strap-shaped and branching, the growth being principally localized at one end.

Perithecia are produced beneath the thallus as local circular thickenings. They dehisce by means of a pore torn in the thallus above the centre.

The first species described was *Asteroma Labecula* Montagne (1840), from material collected in South America. Later (Montagne, 1856) it was transferred to the genus *Asterina*. In 1868 a fungus collected in Cuba was described by Berkeley and Curtis as *Asterina reptans*. Spegazzini (1889), from an examination of the material used by Berkeley and Curtis, recognized a fungus of distinctive character, which he took to be that described by the earlier authors. Because of its outstanding differences from the genus *Asterina* he placed it in a new genus *Trichopeltis*, using the specific name *reptans* of Berkeley and Curtis. It seems clear from the descriptions given, however, as Stevens (1925) has pointed out, that the type of *Asterina reptans* B. & C. is not the same fungus as that described as *Trichopeltis reptans* (B. & C.) Speg. by Spegazzini. Consequently it is doubtful whether all the fungi recorded from various parts of the world as *Asterina reptans* belong to the genus *Trichopeltis*.

Spegazzini also (1888) described the genus *Brefeldiella* which has a flat circular thallus, one cell thick. The genus *Trichopeltella*, very similar to *Trichopeltis*, was described by von Hoehnel in 1910.

Theissen (1913) proposed the family Trichopeltaceae to include these genera and described several new genera based on characters of spore septation and colour. He placed *Asterina Labecula* in the genus *Trichopeltis*, and gave a very detailed account of the structure and growth of the thallus in this family.

Stevens (1925) gave a review of the family and species described. He clarified the position with regard to the nomenclature of *Trichopeltis* and *Asterina reptans*, and gave conclusive reasons for the acceptance of *T. reptans* Speg. as the type of the family. He rejected *Asterina reptans* B. & C. as a fungus too imperfectly described to be recognizable.

At present the family Trichopeltaceae is regarded as being closely allied to the Microthyriaceae.

b. Species occurring in New South Wales.

Cooke (1892, p. 315) recorded the fungus *Asterina reptans* B. & C. as occurring on leaves in Queensland. The description he gave is as follows: "Mycelium thin, rather reticulated, perithecia minute, constructed from the radiating cells, asci clavate, sporidia oblong, somewhat fusiform, uniseptate." From this, which is a direct translation of the type Latin description given by Berkeley and Curtis, it is impossible to tell without an examination of the original material whether an *Asterina* or a *Trichopeltis* is indicated.

Theissen (1913), in the course of his investigation of the family Trichopeltaceae, examined specimens from the Kew collection labelled *Asterina reptans*. He made no mention of any specimen from the mainland of Australia; therefore, it seems probable that the fungus recorded by Cooke is a species of *Asterina*, not *Trichopeltis*. Theissen recorded *Trichopeltis reptans* Speg. on the leaves of *Tasmania* (probably a typographical error for *Drimys*) *aromatica*, Tasmania, from collections at Kew.

No other record has been made of the occurrence of this fungus in Australia.

During the present investigation three species belonging to the Trichopeltaceae have been collected.

1. *Trichopeltis reptans* Speg.—This species has been identified in 45 collections. It is most common on the leaves of rain-forest trees in shaded localities, either alone or associated with sooty-mould fungi, chiefly members of the Chaetothyrieae (Plate xiii, fig. 1). The thallus is strap-shaped, branching at intervals, closely adherent to the host leaf (Plate xiii, fig. 2). The cells in the centre of the thallus form a longitudinal strand. In old thalli the cells along the margin may grow outwards at right angles to the long axis, thus giving rise to a certain amount of lateral growth (A in fig. 2, Plate xiii). *Trichopeltis reptans* appears to be a widespread species. It is common in South America and the West Indies, and has also been recorded in the East Indies and in Hawaii.

2. *Brefeldiella brasiliensis* Speg.—This species has been identified in 20 collections. Like *Trichopeltis reptans*, it is found chiefly on the leaves of rain-forest trees associated with *Atichia* and the Chaetothyrieae (Plate xiii, fig. 3). The thallus is roughly circular or slightly lobed (Plate xiii, fig. 4). The margin is more or less even. The fungus grows smoothly round obstacles in its path, such as a colony of *Phycopeltis* or a young *Brefeldiella*, joining up again on the other side. Consequently an old thallus may have a patchy appearance. *Brefeldiella brasiliensis* has been recorded in South America.

3. *Trichothallus hawaiiensis* Stevens.—This species has been identified in three collections. It therefore appears to be much less common than the two preceding species. It occurs on the leaves of rain-forest trees in especially humid situations, associated with *Trichopeltis* and members of the Chaetothyrieae (Plate xiii, fig. 5).

It was first described by Stevens (1925) from Hawaii, and has not been recorded since. As originally described, the fungus showed neither pycnidia nor perithecia, and none were observed in the material collected in New South Wales. From the structure of the thallus it is obvious that the fungus belongs to the Trichopeltaceae. The margin of the thallus is, as a rule, rather uneven. Not uncommonly a single hypha, or several hyphae together, may grow out as a narrow strand. The forward growing edge also sometimes shows this feature, single hyphae, or narrow bands of hyphae growing out, often later coalescing, thus giving the thallus a reticulate appearance (Plate xiii, fig. 6). The most peculiar feature of the thallus is the presence of hairs. These are single hyphae composed of cells growing upwards from the thallus at frequent intervals. They measure 75–120 × 9–10μ, often tapering towards the base and apex. They break off fairly readily and undoubtedly function as organs of propagation.

Because of its more loosely aggregated thallus and the presence of hairs, this species appears to be a more primitive type than either *Trichopeltis* or *Brefeldiella*.

Trichothallus hawaiiensis has been collected on the leaves of *Backhousia myrtifolia* and *Eugenia Smithii* in the Upper Williams River District, N.S.W., May 1933, January 1934, and May 1936.

The Atichiaceae.

A review of the changes in nomenclature and classification of the members of this family has been given in an earlier paper (Fraser, 1932).

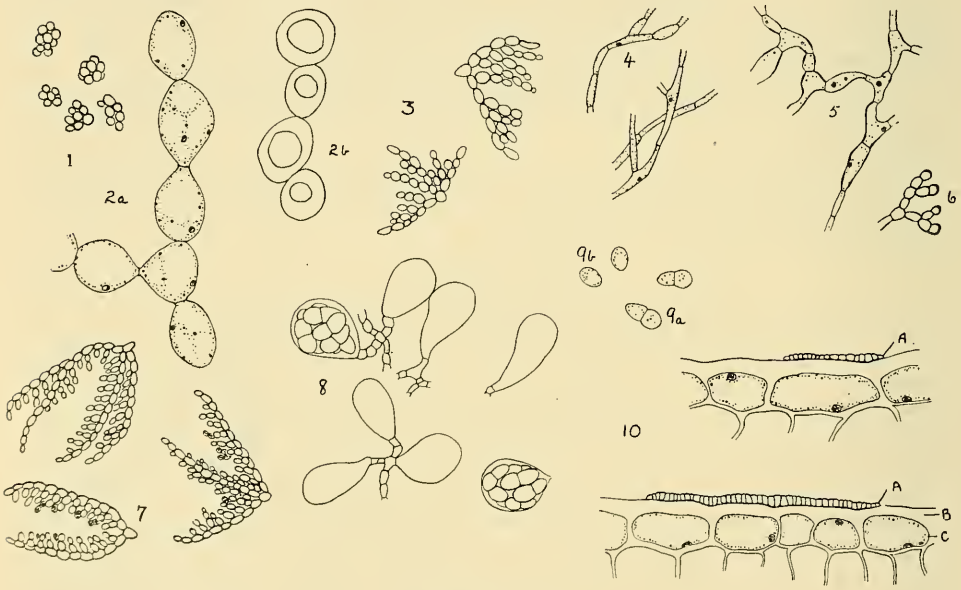
Only two references have been made to the occurrence of *Atichia* in Australia. McAlpine (1896), in describing the sooty-mould-forming fungus *Capnodium citricolum* attributed to it structures which he called "glomerulae", and which he considered to be a type of imperfect fructification. These were globular gelatinous colonies of yeast-like cells, 0·5 mm. or more in diameter. Through the kindness of the Government Biologist of the Department of Agriculture, Victoria, the writer was able to make an examination of McAlpine's type specimens. Small colonies of *Atichia glomerulosa* were recognizable in one of his collections, and there can be no doubt that these are the "glomerulae" to which he referred. Fisher (1932) recorded a species of *Phycopsis* on *Myoporum* and *Bursaria* in Victoria, but was unable to assign it to any species owing to the lack of perfect material.

Four species of fungi belonging to the Atichiaceae have been found in collections made during the present investigation.

1. *Atichia glomerulosa* (Ach.) Flot.—This species has been identified in 17 collections. It is usually associated with sooty mould fungi, and occurs both in sunny and shaded positions. The colonies are globular and gelatinous, composed of yeast-like cells, usually 300–600μ in diameter, mostly closely aggregated, or solitary, sometimes obscurely lobed. Somewhat stellate colonies up to 2 mm. in diameter consisting of a number of large lobes have been seen. The propagulae, which are borne in a concavity at the apex of the thallus, are globular or obscurely 3-branched, and 12–20μ in diameter (Text-fig. 1). The asci, which are borne just below the surface at the apex of the thallus, are globular or pear-shaped, and

8-spored. The ascospores are 1-septate, hyaline, 12–16 × 8μ. This agrees very well with the descriptions given by Vuillemin (1905) and von Hoehnel (1910), and with the figures of Arnaud (1910). This species appears to be very widespread; it is common in Europe and has been recorded in many countries, including Java.

2. *Atichia Millardeti* Pat.—This species has been identified in 58 collections, and appears to be by far the most common *Atichia* in New South Wales. It occurs exclusively in shaded positions, chiefly on the leaves of rain-forest trees, either alone or associated with members of the Trichopeltaceae and Chaetothyriaceae (A in fig. 7, Plate xiv). The colonies have always 3 or more prominent lobes (Plate xiv, figs. 8, 9) and may attain a diameter of 5–6 mm. They are composed of yeast-like cells (Text-figs. 2*a*, 2*b*). The propagulae are produced in elongated or circular cavities in the upper surface of the lobes of the thallus (A in Plate xiv, fig. 9). They are triquetrous branch systems, 40–50μ in length



Text-figures 1-10.

1.—Propagulae of *Atichia glomerulosa*. × 285.

2.—Cells of the thallus of *Atichia Millardeti*, 2*a* showing cytoplasm and nuclei after removal of oil, 2*b* showing oil drops. × 1,000.

3.—Propagulae of *Atichia Millardeti*. × 285.

4-9.—*Atichia botriosa*.

4.—Hyphae from the centre of the lobes of the thallus showing filamentous nature. × 285.

5.—Hyphae from nearer the margins of the lobes of the thallus than those shown in Text-figure 4, showing slight inflation and irregular darkening of the walls. × 285.

6.—Hyphae from the margins of the lobes of the thallus showing thickening of the walls, branching and bead-like appearance. × 285.

7.—Propagulae showing branching. × 285.

8.—Asci. × 285.

9*a*.—Ascospores; 9*b*.—Cells of the ascospore after separation. × 285.

10.—Part of two transverse sections of the epidermis of a leaf showing the thallus of *Trichopeltis reptans* (A). B, cuticle; C, epidermal cells. × 285.

(Text-fig. 3). The asci are produced in raised cushion-like swellings near the base of the lobes of the thallus (A in Plate xiv, fig. 10). They are very numerous, globose or pear-shaped and 8-spored. The ascospores are 11–14 × 6–7μ, 1-septate, hyaline, slightly constricted at the septum.

The branches of the propagulae of the type specimens of *A. Millardeti* are described as being terminated by narrow hair-like cells. Such hairs are not a feature of the New South Wales form, but thin cells approximating to them have been seen several times. However, the New South Wales form resembles *A. Millardeti* so closely in all other respects that it is regarded as a climatic variation only of the type. *A. Millardeti* has previously been recorded only from Tahiti.

3. Atichia botriosa, n. sp.—Thallo gelatinoso, ramosissimo, ad 5 mm. lato et ad 4 mm. crasso, nigro arido, fusco humido, ramis brevibus et latis. Thalli hyphis in muco immersis, ramorum mediis partibus angustis et hyalinis, margine moniliformibus, fuscis, stipitatis. Propagulis fuscis, in lacunis ramorum apicibus latis, 1–5-, plerumque 3-ramis, 55–75 × 10–14μ. Ascis globosis aut piriformis, fere sub superficiem ramorum apicibus latis, 30–42 × 18–25μ, 8-sporis. Sporis hyalinis, 1-septatis, septo constrictis, 16–19 × 7–8μ, altera cellula paulam longiore et angustiore et acutiore altera. Cellulis aliquando etiam in asco separantibus.

The colonies are epiphyllous, gelatinous, up to 5 mm. in diameter and 4 mm. in thickness. They are black when dry, dark sooty-brown when moist, adhering firmly by means of a large gelatinous disc. The thallus is closely branched in three dimensions in a somewhat dichotomous fashion, forming a botryoidal colony (Plate xiv, fig. 11). The branches or lobes are as broad as long, and are rounded at the apex (Plate xiv, fig. 12). The thallus is composed of hyphal threads embedded in mucilage. The hyphae at the centre of the lobes are filamentous, narrow, thin walled and light coloured, the cells measuring 40–50 × 2–6μ, not, or scarcely, inflated (Text-fig. 4). Towards the periphery the hyphae branch and anastomose fairly frequently; the cells are shorter, broader and somewhat constricted at the septa, 15–30 × 6–10μ, light coloured but with parts of the wall thicker and darker (Text-fig. 5). The cells forming the outer layer of the thallus are bead-like, globular or nearly so, constricted at the septa but not yeast-like, 4–6μ in diameter, the walls moderately thick and dark, especially the outermost wall of the apical cell of each filament (Text-fig. 6). The central hyphae are arranged longitudinally in the thallus; towards the outside they branch in all directions, and at the margin are arranged in close, dichotomously branching, parallel rows at right angles to the surface of the thallus. The propagulae are dark brown, produced in very great numbers in cavities at the apices of the lobes (A in Plate xiv, fig. 13); when full grown they are 1–5-, usually 3-branched, the branches comparatively long and narrow, 55–75 × 10–14μ (Text-fig. 7). Each cell of the branch bears on its inner side 1 or 2 rows of 2–3 small cells. Asci are produced in considerable numbers at the ends of the lobes, just below the surface. They are globular to pear-shaped, 8-spored, 30–42 × 18–25μ (Text-fig. 8). Asci and propagulae are not produced simultaneously. The ascospores are hyaline, 2-celled, constricted at the septum, one cell relatively longer, narrower and more pointed than the other, 16–19 × 7–8μ (Text-fig. 9*a*). The cells often separate while still in the ascus, becoming ovoid and vacuolate (Text-fig. 9*b*).

Atichia botriosa is distinguished from all other species of this genus by the number, size and shape of the branches of the propagulae, and by the shape and method of branching of the thallus, and by the filamentous nature of the cells composing it.

Atichia botriosa has been collected at Barrington Tops, on *Callistemon pallidus* D.C., January, 1934 (asexual stage), October, 1934 (asexual stage), and March, 1934 (sexual stage).

4. *Phycopsis vanillae* (Pat.) Mang. & Pat.—This species has been identified in 9 collections. It is usually associated with sooty-mould fungi and occurs only in shaded positions (A in Plate xiii, fig. 3). The colonies are globular, 200–400μ in diameter, never lobed, and composed of yeast-like cells. The propagulae, which are large and globular, are produced singly all over the upper part of the thallus, just below the surface. The asci are borne in the thallus just below the surface as in *Atichia glomerulosa*. They are globular or pear-shaped, and 8-spored. The ascospores are 1-septate, hyaline, 15–18 × 7–9μ. *Phycopsis vanillae* has been recorded in Java, Tahiti and the Gambier Islands.

Mode of Nutrition.

It has been recognized from the first that the species of the Atichiaceae are saprophytes, but their dependence on scale-insect excretions has not previously been established. Any reference to the source of food has usually been omitted in descriptions of species. Cotton (1914) noticed the occasional association of *Atichia dominicana* with scale insects, but came to the conclusion that it was without significance. However, as he dealt only with dried material, his conclusions were not based on a first-hand knowledge of the conditions under which the fungus lived. In the writer's experience, *Atichia* mostly occurs where scale insects are present, and is usually associated with members of the Capnodiaceae. Colonies of *Atichia* have sometimes been found in positions where they would receive a little "honey dew", but are not directly associated with scale insects. For instance, the scale insects might be on a neighbouring tree. The most numerous and luxuriant colonies have always been found nearest to parts affected by scale insects. They have never been found in any position where scale insects are entirely absent.

In the case of the Trichopeltaceae the position is not so clear. Neither Spegazzini (1889) nor Theissen (1913) has expressed any opinion on the mode of nutrition of the members of this family. In discussing *Trichopeltis reptans* Stevens (1925) used the word "host" when speaking of the tree on whose leaves this fungus grew. This seems to indicate that he considered the species to be parasitic. From observations in the field the writer came to the conclusion that the members of the Trichopeltaceae formed part of the sooty-mould association. A careful examination of the relation of the thallus of the various species of the Trichopeltaceae to the leaves on which they occurred was therefore necessary. The following points were observed: The thallus was found to lie in very close contact with the epidermis of the leaf on which it grew, following its contours exactly. Microtome sections 4μ thick were cut of parts of leaves bearing *Trichopeltis* and *Brefeldiella*. No trace of haustoria could be found (Text-fig. 10). The thallus of the fungus appears to be strictly external to the cuticle. No case has been observed of damage by the species of the Trichopeltaceae to the leaf on which they were growing. This in itself does not mean that they are not parasitic, since species of *Meliola*, though parasitic, sometimes appear to do no visible harm to their host leaves. An additional proof of their saprophytic nature is afforded by the fact that dry thalli of *Trichopeltis*, *Brefeldiella* and *Trichothallus* can be flaked off the leaves on which they grow. This is well shown by *Brefeldiella*, in which the central parts of large colonies often break off, leaving the margins to continue their growth (C in Plate xiii, fig. 3). It was found that both *Brefeldiella*

and *Trichopeltis* grow readily in culture on potato-dextrose-agar. These facts seem to afford sufficient proof that the members of this family are not parasitic.

In the field, the three species of the Trichopeltaceae are frequently associated with sooty moulds. The best development takes place near scale insects. Often, however, their thalli appear to be unassociated with scale insects or other fungi (Plate xiii, fig. 1). In these cases scale insects are always to be found on neighbouring trees, so that a little "honey dew" is probably available to the fungi. Moreover, small scale-insects of a brown or greenish colour, closely adhering to stems or the under-surfaces of leaves, are easily overlooked.

On the above grounds it appears reasonable to assume that the members of the Atichiaceae and Trichopeltaceae are true saprophytes living on "honey dew" like the members of the Capnodiaceae, and that they therefore should be included in the sooty-mould group.

Systematic Position of the Atichiaceae.

The family Atichiaceae has been considered by various writers to show affinities with the Saccharomycetes, Capnodiaceae, Bulgariaceae, etc. The general opinion seems to be that it is most closely related to the Saccharomycetes because of the yeast-like appearance of the cells composing the thallus.

In this connection it may be of interest to consider the species *Atichia botriosa*. This is a typical member of the Atichiaceae in all respects, except in the nature of the mycelium. Instead of being composed of yeast-like cells easily separating from each other, the hyphae, especially those at the centre of the thallus, are filamentous, not inflated. Each cell contains one small inconspicuous nucleus (Text-figs. 4, 5), quite unlike the large characteristic nucleus of the yeasts. This feature is also shown by *A. Millardeti* (Text-fig. 2*a*).

Asci in all members of the Atichiaceae occur embedded separately in specialized areas in the thallus as in the Myriangiales and appear to be produced by a ramifying system of ascogenous hyphae (Text-fig. 8).

It therefore seems probable that the relationships of the Atichiaceae with the Saccharomycetes are apparent rather than real, and that its closest relationships are with the Myriangiales.

Summary.

A brief review is given of the changes in nomenclature and classification of the members of the Trichopeltaceae.

Trichopeltis reptans Speg., *Brefeldiella brasiliensis* Speg. and *Trichothallus hawaiiensis* Stevens are recorded for the first time in New South Wales. *Atichia glomerulosa* (Ach.) Flot., *A. Millardeti* Pat. and *Phycopsis vanillae* (Pat.) Mang. and Pat. are recorded for the first time in New South Wales. A new species, *Atichia botriosa*, is described.

It is shown that both the Atichiaceae and Trichopeltaceae are epiphytes dependent on scale-insect excretions, and that they therefore form part of the sooty-mould flora.

Because of the position of the asci and the nature of the mycelium, especially in *Atichia botriosa*, the Atichiaceae are considered to show affinities with the Myriangiales.

In conclusion, the writer wishes to thank Assistant Professor J. McLuckie, of the Department of Botany, University of Sydney, for helpful criticism during the course of this work.

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* Abstracts only available to the writer.

EXPLANATION OF PLATES XIII-XIV.

Plate xiii.

1.—Leaves of *Eugenia Smithii* showing thalli of *Trichopeltis reptans* Speg. × ½.

2.—Part of the thallus of *Trichopeltis reptans* showing lobes, marginal growth along edges of lobes (A), and position of the young perithecia (B). × 40.

3.—Leaves of *Citrus* sp. showing colonies of *Phycopsis vanillae* (A) and *Brefeldiella brasiliensis* (B). C, colonies of *Brefeldiella* from which the central parts have flaked off. × ½.

4.—Thallus of *Brefeldiella brasiliensis* showing position of perithecia (A). × 20.

5.—Leaves of *Backhousia myrtifolia* showing thalli of *Trichothallus hawaiiensis* Stevens. × ½.

6.—Part of the thallus of *Trichothallus hawaiiensis* showing setae (S) and method of branching. × 20.

Plate xiv.

7.—Leaf of *Daphnandra micrantha* showing epiphyllous colonies of *Atichia Millardeti*. × ½.

8.—A large colony of *Atichia Millardeti* showing lobing. × 20.

9.—Colonies of *Atichia Millardeti* showing lobing, propagula cavities (A) and disc of attachment (B). × 20.

10.—A colony of *Atichia Millardeti* showing ascal cushions at the base of the lobes. × 20.

11.—Colonies of *Atichia botriosa* on a twig of *Callistemon pallidus*. Nat. size.

12.—Part of a large colony of *Atichia botriosa* showing lobing. × 20.

13.—Part of a colony of *Atichia botriosa* showing lobing and position of the propagula cavities (A) at the apices of the lobes. × 20.